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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Maino et al.
Serial No. : 08/803,702
Filed : February 21, 1997
For : METHOD FOR DETECTING T CELL RESPONSE TO
SPECIFIC ANTIGENS IN WHOLE BLOOD
Group Art Unit : 1644
Examiner : Gerald Ewoldt, Ph.D.

Bethesda, MD

Hon. Commissioner for Patents
Washington, D.C. 20231

SECOND DECLARATION OF CALMAN P. PRUSSIN, M.D.
UNDER 37 C.F.R. § 1.132

Sir:

I, CALMAN P. PRUSSIN, M.D., declare as follows:

1. My credentials are set forth in an earlier declaration, dated November 20, 2000 (my "first declaration"), which I understand to have been filed shortly thereafter in support of the present application.

2. I have been asked to elaborate on my conclusion, as set forth in paragraphs 35 - 42 of my first declaration, that "the claimed methods of detecting antigen-specific T

lymphocytes can, without undue experimentation, be practiced by detecting other than the explicitly named cytokines."

3. In my first declaration, I noted that neither Jung et al., Picker et al., nor we ourselves – the three research groups that had, prior to December 1996, used flow cytometry to detect cytokines within T lymphocytes – had to raise its own antibodies. As early as Jung's work in 1993, anti-cytokine antibodies were readily available; the problem, for the most part, was simply one of selecting from among available antibodies, by routine screening, those that could be used for intracellular detection of cytokines.

4. Among the anti-cytokine antibodies that would have been readily available for routine screening in late 1996, early 1997, many had already been shown to be useful for detecting intracellular cytokines, albeit by slide-based immunohistochemistry or immunofluorescence, rather than by flow cytometry. Andersson et al.¹, for example, had demonstrated as early as 1994 that there existed at least one antibody for each of 19 different cytokines that could be used for intracellular detection; although the detection method differs from the flow cytometric methods described in the present patent application, the technique for intracellular staining is not dissimilar. Thus, I would expect that the skilled artisan would have included such antibodies among those to be screened in order to increase the probability of identifying an antibody useful for detection of intracellular cytokines by flow cytometry.

¹ Andersson et al., "Concomitant *in vivo* production of 19 different cytokines in human tonsils," *Immunology* 83:16 – 24 (1994) (attached to my first declaration).

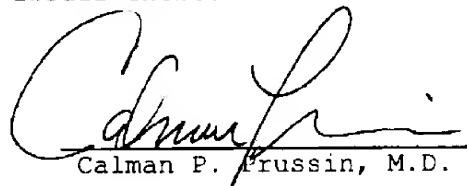
5. Our own experience demonstrates that selecting antibodies useful for the flow cytometric detection of intracellular cytokines would have been attended by a high rate of success, notwithstanding any theoretically postulated difference in conformation as between secreted and intracellular cytokines. In early work performed in 1993 and 1994, we examined hybridoma panels against IL-2 (ten unique clones) and IL-4 (five unique clones) for the ability of the monoclonal antibodies they produced to recognize intracellular cytokines. All ten of the anti-IL-2 clones and 4 of the 5 anti-IL-4 clones were able to stain intracellular cytokine.

6. I believe that others of skill in the art, in order to practice the methods described and claimed in the present patent application with other than the exemplified cytokines, would have followed a similar approach - obtaining three to four distinct antibodies already known to be specific for the chosen cytokine, particularly including those known to be effective for intracellular staining, substituting each in turn for the exemplified TNF- α , IL-2, and IFN- γ antibodies in the described methods, and observing which provided the best results - and would have had similar high rates of success. It would then have been simply a matter of routine to optimize the signal-to-noise ratio for each such positive antibody by varying the antibody and antibody concentration. Given the high level of skill in the art, I would venture that such optimization would have been routine for most, if not all, cellular immunologists by late 1996.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and

further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and may jeopardize the validity of the application or any patent that issues thereon.

7/30/01
Date


Calman P. Prussin, M.D.